A SURVEY OF MICROBIAL CONTAMINATION OF MAPLE SAP IN FIELD COLLECTION SYSTEMS

Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

ABSTRACT

A 3-year study of the sanitary quality of sap collected from buckets and plastic tubing systems showed little difference in bacterial counts in sap collected by either method. Higher bacterial counts were noted in sap samples collected in buckets from an area having a high level of dust pollution and in samples from poorly drained tubing on level terrain. Mobile hauling tanks collecting sap from bucket systems and stationary tanks collecting sap from tubing systems were major sources of bacterial contamination. However, a sanitation program maintained the tanks in good sanitary condition. Bacterial populations were never large enough to affect sirup quality adversely.

A SURVEY OF MICROBIAL CONTAMINATION OF MAPLE SAP

IN FIELD COLLECTION SYSTEMS

Lloyd Sipple, * J. C. Kissinger, 7 and C. O. Willits *

INTRODUCTION

The texture, color, and flavor of maple sirup are frequently determined by the sanitary quality of the raw sap from which sirup is made. Though maple sap is sterile as it flows from the tubules of the tree, microbial contaminants introduced into the sap during the interval between sap flow and atmospheric boiling can cause the sirup produced to have off-flavors, dark color, ropy texture, or all three. $(7)^{\frac{1}{7}}$ Consequently, the maple producer is continually faced with the problem of preserving raw sap from microbial degradation during harvest and storage.

In recent years, the development of the central evaporator plant has modernized the maple sirup industry, and technical advances have done much to minimize losses from sap spoilage. For example, the development of the germicidal taphole pellet has diminished the levels of contamination contributed to raw sap from foci in tapholes. Furthermore, the pellet has increased sap yields by preventing premature stoppage of sap flows caused by formation of microbial slime plugs in the tubules of the tree.(2, 5) Plastic tubing sap collection systems are now used to gather more than 40 percent of the sap harvested in the United States. This innovation requires less labor than the traditional bucket collection system and reduces the time needed to convey sap from the tree to the evaporator house.(8) In addition to these developments, the use of germicidal ultraviolet lights has enabled the evaporator plant operator to store raw sap in tanks for up to 5 days without losses from microbial action.(6)

As the maple sirup industry progressed, it became apparent that little was known about the occurrence of microbial contaminations in maple sap or the influence of sap collection techniques on the sanitary quality of sap. Small scale studies indicated that plastic tubing could be used for sap harvesting without endangering the sanitary quality of sap. (3) A subsequent study, made during a late season sap run, gave evidence that sap collected from tubing systems contained lower levels of bacterial and yeast contaminants than that gathered from bucket collection systems. (4) However, these studies were not conducted in a manner which would provide sanitation data representative of an industrial-scale operation throughout a complete maple season. The research reported herein was conducted to determine which method of collecting sap (buckets or plastic tubing) allowed the greater amount of microbial contamination.

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Numbers in parentheses refer to Literature Cited, p. 10.

MATERIALS AND METHODS

Bacterial and Yeast Counts

All bacterial and yeast plate counts were made in a manner similar to American Public Health Association standard methods.(1)

Tapping Procedure

All trees were tapped in accordance with the standard practices in current use by the evaporator plant personnel. Holes were drilled to a depth of 3 to 4 inches with a power drill fitted with a 7/16-inch bit. One paraformaldehyde germicidal pellet was placed in each taphole and a sanitized spile was placed in the taphole.

Sap Collection Sites and Collection Equipment

- 1. Three sugar bushes equipped with buckets were used as sampling points in these studies. The sugar-bush designations and locations were as follows:
 - Bush 1: A natural growth of trees with diameters at breast height (d.b.h.) 24 to 30 inches located on the south side of a hill.
 - Bush 2: A row of planted trees with d.b.h. 24 to 30 inches located beside a blacktop road.
 - Bush 3: An orchard of planted trees with d.b.h. 24 to 30 inches located beside a farmhouse >100 feet from a road.
- 2. Three sugar bushes equipped with plastic tubing sap collection systems were selected as sampling sites. The sugar-bush designations and locations were as follows:
 - Bush 1: A group of planted trees with d.b.h. 20 to 30 inches located on level ground. There were 120 taps using 5/16-inch tubing. All ground lines remained full of sap at all times.
 - Bush 2: A naturally grown, unimproved sugar bush located on the south side of a hill with a grade varying from 10 to 40 percent. There were 300 taps on trees with d.b.h. 10 to 30 inches. Three hundred feet of 1/2-inch well-drained pipeline was used to transport the sap to the roadside tank.
 - Bush 3: A naturally grown, improved sugar bush located on the east side of a hill with a grade varying from 10 to 40 percent. There were 1,100 taps on trees with d.b.h. of 10 to 24 inches. Two thousand feet of 1/2-inch and 3/4-inch well-drained pipeline was used to transport sap to the roadside tank.

- 3. Three truck-mounted tanks were used to transport to the evaporator house sap gathered from the buckets.
- 4. In the sugar bushes equipped with plastic tubing systems, three stationary tanks were used as holding vessels for sap effluent from the main conduit lines.

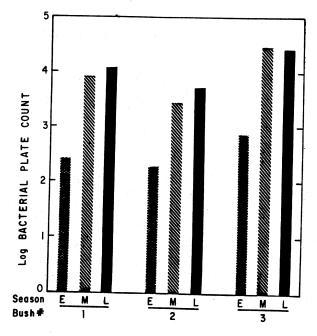
Sampling Techniques

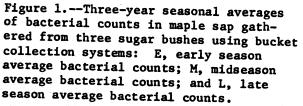
Sap samples were taken on each sap flow day using aseptic techniques. The temperature of each sample was recorded at the time the sample was taken, and the pH was measured when the sample was delivered to the laboratory. Samples from the bushes using bucket collection systems were prepared by taking 4 ounces of sap from one bucket at each of 10 randomly selected trees in each bush. The 10 samples were composited to provide one representative sample per collection site. One sap sample was taken from the main conduit effluent at each sugar bush using plastic tubing for sap collection. Samples were taken from the drop valves of both the stationary tanks and the mobile tanks.

RESULTS AND DISCUSSION

All data gathered during the three maple seasons included in this study are presented in tabular form in the appendix. During this period, yeast counts were consistently low and never approached the magnitude of bacterial counts made on identical samples. The low yeast populations are a result of the speed with which the sap was gathered and the basic field sanitation program followed by the evaporator plant personnel. However, these consistently low yeast populations indicate that a well-managed sap collection operation can so minimize the effects of slow growing yeast contaminants that they have little opportunity to degrade sap and thereby lower the quality of the sirup. Bacterial counts on samples taken during this study varied widely and provided a good basis for comparing the different collection techniques used.

Figure 1 shows the 3-year average bacterial plate counts made on sap collected in buckets during early-season, midseason, and late-season sap flows at the three sugar-bush locations studied. Each bar on the graph represents the logarithm of the average bacterial count for the designated sampling location and sap flow period. The sanitary quality of the sap gathered from all three sugar bushes was quite good, and there was no indication of unsanitary conditions which might have had an adverse effect on sirup quality. The average bacterial counts from bushes 1 and 2 showed typical early- to late-season increases reflecting the progressively higher ambient temperatures of the maple season. But bacterial counts made on samples taken from bush 3 were, on the average, threefold to fourfold higher than those of the other bushes. Since tapping procedures were identical, and all of the buckets were equipped with the same type of cover, it appears that the higher counts can be attributed to the location of the sugar bush. Bush 3 was adjacent to a barnyard which housed more than 20 dairy cattle. In addition, a dirt road, frequently used by farm vehicles, ran from the main paved road to the barn area. This research was done during several seasons when rainfall was far below normal, and bush 3 was subject to more dust contamination than bushes 1 and 2, because of its proximit to the barnyard and dirt road. Thus, the higher levels of bacterial growth





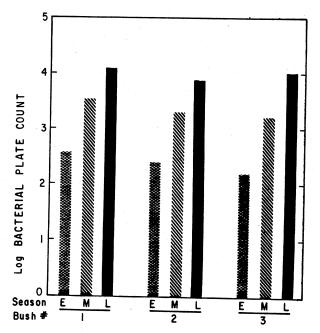


Figure 2.—Three-year seasonal averages of bacterial counts in maple sap gathered from three sugar bushes using tubing collection systems: E, early season average bacterial counts; M, midseason average bacterial counts; and L, late season average bacterial counts.

found in sap from bush 3 probably are a result of air-borne contaminants rather than defects in sap collection techniques.

Figure 2 shows the 3-year average bacterial plate counts of sap samples taken from the tubing line effluent streams of the three bushes using plastic tubing for sap collection. The bacterial counts were low and gave no indication of unsanitary conditions which might have had an adverse effect on sirup quality. The increase in average counts from early to late season was typical and almost uniform for the three bushes. In every instance, the average counts from bush 1 slightly exceeded those from the other two bushes. This variation was due to the level terrain of bush 1 where the tubing lines remained full of sap at all Some bacterial growth took place in residual drops of sap in the welldrained tubing lines, but the tubing lines filled with sap provided a better environment for bacterial growth. A comparison of the sanitary quality of sap gathered by the two collection methods (buckets vs. tubing) shown in figures 1 and 2 gives no indication that one method was superior to the other from the standpoint of sanitation under existing conditions. In bush 3 where sap was collected by the bucket method, a tubing collection system could possibly have provided sap of better sanitary quality by excluding most of the air-borne contaminants introduced from road and barnyard dust. Thus, augar-bush location and terrain are points to consider when choosing a sap collection method.

The 3-year average bacterial counts of sap delivered to the plant in hauling tanks from bucket collection systems of bushes 1, 2, and 3 are shown in figure 3. These counts show a fivefold to tenfold increase in bacterial population over the counts of the corresponding sap composite samples (fig. 1) taken

from the collection buckets of the three sugar bushes. This demonstrates the importance of the hauling tank as a source of contamination in sap handling. The early season counts averaged <1.1 x 104 per ml. reflecting the initially good sanitary condition of the equipment and low seasonal temperatures. At the end of each day's operation, the tanks were rinsed with tapwater and drained. This procedure failed to maintain the hauling tanks in good sanitary condition as the season progressed, and midseason average counts showed a buildup of bacterial growth in sap delivered in tanks 1, 2, and 3 resulting in counts of 3.9×10^5 per ml., 7.5×10^5 per ml., and 2.0×10^5 per ml., respectively. increasing levels of contamination in the collection buckets, warmer temperatures, and the deterioration of tank sanitation decreased the sanitary quality of the sap. When the increased bacterial counts were noted, the hauling tanks were sanitized with a 0.5 percent sodium hypochlorite solution. The effect of this treatment is shown by the decreases in average bacterial counts in delivered sap to 1.9 x 10^5 per ml. (tank 1), 2.2 x 10^5 per ml. (tank 2), and 1.1 x 10^5 per ml. (tank 3) during late season sap run. If the hauling tanks had not been sanitized, the bacterial counts of these late season sap flows probably would have shown increases corresponding to those observed in the composite samples taken from buckets during the same sap flow period (fig. 1).

The 3-year seasonal average bacterial counts of sap gathered in stationary tanks from the plastic tubing collection systems of bushes 1, 2, and 3 are shown in figure 4. Sap samples taken from tank 1 had higher bacterial counts than corresponding samples from tank 2. Tank 1 received sap from the tubing system installed on level ground. The highly contaminated sap from this system drained

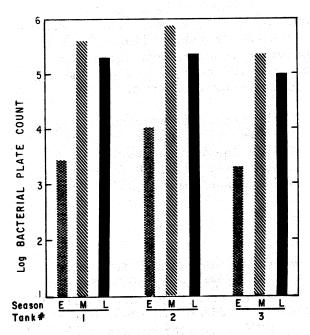


Figure 3.—Three-year seasonal averages of bacterial counts in maple sap gathered in mobile tanks from three sugar bushes using bucket collection systems: E, early season average bacterial counts; M, midseason average bacterial counts; and L, late season average bacterial counts.

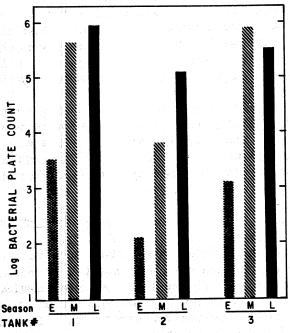


Figure 4.—Three-year seasonal averages of bacterial counts in maple sap gathered in stationary tanks from three sugar bushes using tubing collection systems: E, early season average bacterial counts; M, midseason average bacterial counts; and L, late season average bacterial counts.

into the tank, and the bacterial population of this sap was further increased by organisms from foci in the tank and by the normal growth of bacteria during the holding period before samples were taken. All stationary tanks were sanitized at the end of each sap flow period. Therefore, the higher bacterial counts obtained from tank 1 show that sanitary conditions in a sap gathering tank depend to a great degree on the sanitary quality of the sap received from the tubing lines. Late-season average counts made when climatic conditions favored bacterial growth reached 1.2 x 10^5 per ml. in tank 2, while corresponding samples from tank 1 averaged 8.9 x 10^5 per ml. The average bacterial counts for tank 3 cannot be used for comparison with the preceding data because the cover of the tank was damaged by vandals, and dirt was thrown into the collected sap during a midseason sap run of the second year. As a result, the midseason average bacterial count of sap in this tank was 7.8 x 10^5 per ml. This high bacterial count clearly demonstrates the effect of soil contamination, deliberate or inadvertent, on bacterial population levels in sap.

A comparison of figure 2 with figure 4 shows that sap samples taken from the gathering tanks in bushes 1 and 2 had higher average bacterial counts than samples taken directly from the sap streams flowing from the plastic tubing lines into the gathering tanks. These higher counts reflect the development of contamination foci in the gathering tanks and growth of bacteria in the sap while being held in the tank. As the season progressed and ambient temperatures increased, rapid bacterial growth took place in the gathering tanks. Early season average counts made on samples taken directly from the tubing lines and the gathering tank of bush 2 show insignificant counts of 2.5×10^2 per ml. and 1.3×10^2 per ml., respectively. But at the season's end, average counts of corresponding samples were 7.1×10^3 per ml. and 1.2×10^5 per ml. The increase in average count noted in samples taken directly from the tubing lines shows a typical seasonal progression, but the much greater increase in average count of samples taken from the gathering tank indicates that the tank served as a good incubator for bacterial growth as the ambient temperature increased.

Bacterial counts in the tubing systems' gathering tanks were quite low and never approached a magnitude which would have adversely affected product quality. This was due largely to the program of tank sanitation carried on by plant personnel. At the end of the midseason sap flow period, each gathering tank was rinsed thoroughly with a 0.5 percent sodium hypochlorite sanitizer solution.

CONCLUSIONS

This research shows that in a well-managed sugar bush, there is little difference in the sanitary quality of maple sap, whether gathered in buckets or plastic tubing. However, in some situations, there are advantages to be gained by careful selection of sap collection systems. For instance, in areas where heavy dust contamination can be anticipated, plastic tubing systems are better fitted for sap collection; and in flat-land sugar bushes bucket systems are more suitable.

Sap gathering tanks must be sanitized regularly to prevent development of contamination foci which could ultimately affect the quality of sirup made from sap exposed to these foci. Mobile tanks can be maintained in good sanitary

condition by frequent sanitizing, preferably at the end of each day's operation, and stationary tanks can be maintained in good sanitary condition by periodic sanitizing at the end of each sap run.

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APPENDIX

Data amassed from three-year sanitation survey of maple sap collection techniques.

TABLE 1.--Microbiological contamination levels of sap collected with buckets from three sugarbushes, 1965, 1966, and 1967

		Bush No.	1			Bush No.	, 2		Bush No. 3				
Date sample obtained	Yeast	Bacteria	Temp.	pН	Yeast	Bacteria	Temp.	pН	Yeast	Bacteria	Temp.	pН	
	cells/	m1. $\times 10^{-3}$	<u>•</u> F		cells/	$1. \times 10^{-3}$	<u>•</u> F	<u>cells/ml. x 10⁻³</u> •F					
			and the second		EAR	LY SEASON							
1965:	at Tanana				3 4.7		1. A	i i					
March 4	0.34	0.70	58	6.9	0.40	0.02	59	6.9	0.01	1.20	57	7.0	
March 10	.38	.60	52	6.7	.28	.20	46	6.7	.01	2.70	45	6.7	
March 14	.40	.60	52	6.7									
March 15	.20	.30	60	6.4	.32	.02	56	6.4	.07	2.70	60	6.4	
March 17	.47	.50	50	6.7	.64	.21	52	6.7	.20	.80	42	6.7	
March 18	.40	. 50	48	6.5	.05	.30	46	6.4	<.01	.92	48	6.5	
March 19	.51	.61	64	6.7	.17	.20	56	6.6	<.01	2.10	56	6.7	
					MI	DSEASON							
March 23	.10	.40	62	6.7	.04	.31	62	6.7	.02	2.70	60	6.7	
March 26	.08	.10	46	6.6	.18	.58	48	6.6	<.01	1.80	48	6.6	
March 28	.43	.35	58	6.6	.26	.10	56	6.5	.04	2.20	58	6.5	
					LAT	E SEASON	i i i					1.7	
April 1	.51	.10	40	6.4	.67	.05	40	6.4	.10	9.10	58	6.4	
April 2	.57	.10	45	6.6	1.00	.93	42	6.6	.05	3.30	41	6.6	
April 3	.14	.05	50	6.7	.36	.70	48	6.7	.06	.40	50	6.7	
April 4	.28	.21	50	6.5	.19	.77	56	6.6	.02	.60	52	6.5	
April 5	.40	.40	50	6.5	.07	.20	50	6.4	.06	1.10	50	6.4	
April 6	.10	.30	62	6.5	.06	.40	62	6.5	.01	8.40	61	6.4	
April 11	.58	.77	60	6.5	1.80	3.00	60	6.5	.39	16.00	58	6.5	
					EAR	LY SEASON							
1966:		10			(0:	10	42	6.9	.04	1.00	43	6.5	
March 5	.03	.12	43	6.9	<.01	.19 .07	42 41	6.7	.66	.30	43 39	6.7	
March 10	.20	.10	41	6.7	.21	.07	41	0.7	.00	• 30	33	0.7	

March 1		<.01	.04	36	6.6	.02	.06	26					
March 1		.26	<.01	36	6.9	.51	.20	36	6.6	.34	.46	40	6.6
March 1		.21	.04	42	6.2	.12	.10	36	6.9	<.01	.01	36	6.7
March 1	5	.75	.12	37	6.7	.21		45	6.5	.18	.69	43	6.7
March 1	6	.15	.40	37	6.7	.12	.34	35	6.7	.14	.70	36	6.7
March 1	7	.37	.10	38	6.6	.23	.02	39	6.7	.36	.10	38	6.7
March 1	8	.20	.39	60	5.9		1.70	41	6.7	.50	.36	39	6.7
				- 00	3.9	1 .01	.02	61	5.9	.05	.17	57	5.9
Warral O						MII	DSEASON						
March 20 March 29		8.40	19.00	34	6.5	2.20	12.00	34	6.9	3.00	150.00	35	
march Z	9/	2.70	27.00	36	6.5	4.10	4.00	35	6.9	4.40	15.00	35 35	6.9
						T.A.T	TE SEASON			, , , , , , ,	13.00		6.9
April 2	3	1.00	66.00	39	6.5	1.20							
April 3		3.40	3.00	38	6.9	.02	24.00	38	6.5	3.00	32.00	38	6.5
April 4		2.50	.45	40	6.5		.62	38	6.9	2.60	11.00	38	6.9
April 5		. 59	.50	41	6.9	.04	5.00	41	6.5	2.40	24.00	41	6.9
April 6		.32	9.00	45	6.9	.02	3.20	38	6.9	1.60	2.20	38	6.9
April 7	1	2.80	93.00	44	6.9	.06	.54	44	6.9	1.20	5.50	44	6.9
April 8		1.10	18.00	41		3.90	6.00	45	6.9	2.80	46.00	45	6.9
April 9		3.20	25.00		6.9	.40	46.00	40	6.9	.80	130.00	40	6.9
April 10		1.50	82.00	41	6.9	3.00	22.00	43	6.9	2.20	25.00	42	6.9
			02.00	43	6.2	.23	12.00	43	6.9	2.00	5.00	43	5.9
						EARL	Y SEASON						
1967:													
March 12		.01	.03	41	7.0	<.01	.05	4.0					
March 13	<	<.01	.01	35	7.0	.10	.10	43	7.0	.03	.10	43	7.0
March 14	<	.01	.06	42	7.0	< .01	.10	35 42	7.0	.04	.10	38	7.0
								42	7.0	<.01	.07	42	7.0
March 23		06					SEASON						
March 24		.06	.10	34	7.0	.06	.40	34	7.0	.39	2.50	34	7.0
March 25	1	.07	.10	37	7.0	.20	.69	37	7.0	.01	.10	36	
	1	.01	.02	38	7.0	.20	.31	41	6.6	.04	.40	38	7.0
March 26	1	.10	.10	54	7.0	.05	.10	54	7.0	.10	.95	50	7.0
March 27	دا	.11	.26	48	7.0	.08	.19	47	7.0	6.70	29.00		7.0
March 307		.03	.08	54	7.0	.03	.10	53	7.0	.06	4.50	47	7.0
March 31		.20	.31	60	7.0	<.01	.02	61	7.0	.05	4.50	52 61	7.0
							SEASON		,.0	•05	4.30	01	7.0
April 4		.34	.50	40	7.0	<.01	.03	20					
April 5		.11	.22	59	7.0	1.00	2.50	39	7.0	.80	30.00	38	7.0
					 +-		2.30	57	7.0	1.80	68.00	61	7.0

*No sap. *Buckets sanitized before this run.

TABLE 2.--Microbiological contamination levels of sap collected with plastic tubing from three sugarbushes, 1965, 1966, and 1967

		Bush No.	1		na kukulusk	Bush No.	2			Bush No.	3	1984 - 1984 1984 - 1984
Date sample	Yeast	Bacteria	Temp.	рН	Yeast	Bacteria	Temp.	pН	Yeast	Bacteria	Temp.	рH
obtained	cells/m	11. x 10 ⁻³	• _F		cells/	nl. x 10 ⁻³	• F		cells/m	$1. \times 10^{-3}$	• F	
			-20		EAR	LY SEASON		3.4.4.				
1965:					40 01	0 01	70	6.9	0.04	0.04	68	6.7
March 4	0.10	0.10	70	6.9	<0.01	0.01	52	6.7	<.01	1.3	54	6.7
March 10	<.10	1.8	52	6.7	.01	.01	64	6.6	<.01	.30	68	6.6
March 14	.64	.02	65	6.6	<.01	1.5	64	6.7	<.01	.04	62	6.6
March 15	.20	.12	64	6.6	<.01 .01	.10	50	6.4	.03	.03	44	6.4
March 17	.04	.40	50	6.4		.72	58	6.5	<.01	.07	48	6.4
March 18	<.01	1.9	48	6.4	<.01 *	./2	20	0.5	<.01	1.0	60	6.5
March 19	.08	1.3	62	6.6					1 1.02			
					MI	DSEASON					1	
- No 1 22	†x				×		a para di salah		<.01	.01	58	6.7
March 23 March 26	.01	.40	54	6.6	.04	.01	54	6.5	<.01	.02	52	6.6
March 28	.40	.60	60	6.6	.01	.01	65	6.5	<.01	.04	60	6.5
March 20	1				LAT	E SEASON						
. <u> </u>	4			6.5	.20	.01	62	6.5	₹.01	.01	60	6.4
April 1	2.0	.55	62	6.5	.06	.02	60	6.4	.05	.04	60	6.6
April 2	1.1	.90	61	6.5	.02	.05	52	6.5	.02	.04	54	6.7
April 3	.20	1.6	62 58	6.5	.06	.15	60	6.5	.01	.05	60	6.5
April 4	.03	2.3	68	6.4	.07	.11	70	6.4	.01	.01	70	6.4
April 5	.50	2.1 6.2	70	6.4	.10	1.0	70	6.5	.20	.55	70	6.4
April 6	.20	32.0	68	6.5	2.0	14.0	60	6.5	.25	20.0	60	6.5
April 11	2.0	32.0	00	0.5		Y SEASON						
					 			·	1			
1966:		01	25	6.9	<.01	.02	35	6.9	1.2	.32	35	6.9
March 10	12.3	.01	35	6.6	.03	<.01	53	6.6	.3	.20	55	6.6
March 11		.01	52 53	6.9	.01	1.3	56	6.9	.04	.01	58	6.9
March 12		.04	57	6.7	<.01	.03	68	6.7	<.01	<.01	67	6.7
March 14		.07	68	0.7	.01	.03	46	6.7	.04	.02	48	6.7
March 15	•		25	£ 7				6.4	<.01	.10	36	6.4
March 16	•	.02	35	6.7	.03	.02	35	6.4	<.01	.10	36	•

March 17		.04	35	6.2	<.01	.03	39	6.9	<.01	0.7	0.7	
March 18	.08	.20	47	5.9	.03	.03	69	5.9	.04	,07 .05	37 64	6.7
									•••	.03	04	5.9
					MI	DSEASON						
March 26	.30	3.6	34	6.2	.08	5.9	32	6.5	.23	6.0	38	
March 29	.35	15.0	34	5.9	.40	6.0	34	6.2	.50	4.0	35	6.2
				4					1	4.0		6.6
					LAT	E SEASON						
April 2	.02	.22	34	6.5	<.01	.6	35	6.5	.01	.80	38	6 5
April 3	.28	5.0	37	6.5	.02	.4	38	6.9	.03	1.8	43	6.5
April 4	.06	2.8	53	6.5	1.3	1.0	53	6.9	<.01	1.5	43 53	6.9
April 5	.21	. 42	42	6.9	2.6	4.0	42	6.9	.17	3.0		6.5
April 6	.14	.25	43	6.9	2.8	8.7	44	6.9	.41	9.4	40 43	6.9
April 7	1.1	8.0	58	6.9	2.2	61.0	56	6.9	.20	25.0		6.9
April 8	.2	1.6	42	6.9	2.6	52.0	41	6.9	.31	23.0	55	6.9
April 9	.04	30.0	41	6.5	.50	22.0	41	6.9	.30	27.0	41	6.9
April 10	.08	210.0	45	5.9	1.3	14.0	42	5.9	.33		41	6.9
								3.9	.33	170.0	44	5.9
					EARL	Y SEASON						
967:			-	T								
March 12	<10	80	41	6.6	<10	30	43	6.6	< 10	410		
March 13	< 10	600	34	6.2	< 10	20	35	6.6		<10	40	7.0
March 14	<10	10	38	7.0	<10	500	36	7.0	20 <10	<10 60	32	6.2
							30	/ 40 1	< 11U			
										- 00	38	7.0
					MII	SEASON				00	38	7.0
March 23	< 10	10	34	7.0	MII							
March 24	< 10	10 30	34 33	7.0		180	34	7.0	<10	900	35	7.0
March 24 March 25	< 10 < 10	30 60			<10	180 40	34 35	7.0 7.0	<10 <10	900 <10	35 36	7.0 7.0
March 24 March 25 March 26	< 10 < 10 < 10	30	33	7.0	<10 <10 <10	180 40 10	34 35 44	7.0 7.0 7.0	<10 <10 <10	900 <10 <10	35 36 47	7.0 7.0 7.0
March 24 March 25 March 26 March 27	< 10 < 10 < 10 < 10	30 60	33 37	7.0 7.0	<10 <10 <10 <10	180 40 10 10	34 35 44 46	7.0 7.0 7.0 7.0	<10 <10 <10 <10	900 <10 <10 100	35 36 47 46	7.0 7.0 7.0 7.0
March 24 March 25 March 26 March 27 March 30	< 10 < 10 < 10 < 10 10	30 60 <10	33 37 37	7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10	180 40 10 10 70	34 35 44 46 53	7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10	900 <10 <10 100	35 36 47 46 50	7.0 7.0 7.0 7.0
March 24 March 25 March 26 March 27	< 10 < 10 < 10 < 10	30 60 <10 <10	33 37 37 53	7.0 7.0 7.0	<10 <10 <10 <10 <10 <7	180 40 10 10 70 180	34 35 44 46 53 58	7.0 7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10 <10	900 <10 <10 100 100	35 36 47 46 50 63	7.0 7.0 7.0 7.0 7.0
March 24 March 25 March 26 March 27 March 30	< 10 < 10 < 10 < 10 10	30 60 <10 <10 20	33 37 37 53 56	7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10 27 180	180 40 10 10 70 180 <10	34 35 44 46 53	7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10	900 <10 <10 100	35 36 47 46 50	7.0 7.0 7.0 7.0 7.0
March 24 March 25 March 26 March 27 March 30 March 31	<10 <10 <10 <10 10 10	30 60 <10 <10 20 140	33 37 37 53 56 74	7.0 7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10 27 180	180 40 10 10 70 180	34 35 44 46 53 58	7.0 7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10 <10	900 <10 <10 100 100	35 36 47 46 50 63	7.0 7.0 7.0 7.0 7.0
March 24 March 25 March 26 March 27 March 30	< 10 < 10 < 10 < 10 10	30 60 <10 <10 20	33 37 37 53 56	7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10 27 180	180 40 10 10 70 180 <10	34 35 44 46 53 58	7.0 7.0 7.0 7.0 7.0 7.0	<10 <10 <10 <10 <10 <10	900 <10 <10 100 100	35 36 47 46 50 63	7.0 7.0 7.0 7.0 7.0 7.0 7.0

^{*}No sap.

TABLE 3.--Microbiological contamination levels in the mobile tank used to collect sap from buckets, 1965, 1966, 1967

Date		Tank No.	1			Tank No.	2		Tank No. 3				
sample obtained	Yeast	Bacteria	Temp.	pН	Yeast	Bacteria	Temp.	pН	Yeast	Bacteria	Temp.	pН	
00 2021100	cells/	$m1. \times 10^{-3}$	°F		cells/m	1. $\times 10^{-3}$	• F		cells/ml. x 10 ⁻³ °F				
	<u> </u>				EARL	Y SEASON		en de d					
1965:				-							58		
March 5	0.22	0.95	40	6.8	*	11 m 1905 by <u>il</u> e 1			*				
March 12	.09	5.90	64	6.7	0.39	3.20	52	6.7					
March 15	.06	1.50	44	6.4	1.40	3.50	66	6.7	2.10	13.00	60	6.7	
March 17	.17	. 64	42	6.5	.42	9.20	52	6.7	.18	2.30	54	6.6	
March 18	.29	.96	50	6.7	.25	5.00	50	6.4	.25	1.60	46	6.7	
March 19	.43	20.00	46	6.6	2.60	19.00	58	6.6	.20	1.00	58	6.7	
					MII	SEASON							
March 26	1.30	20.00	54	6.7	2.40	37.00	54	6.7	2.10	40.00	48	6.7	
March 27	.80	8.00	60	6.6	5.00	4.80	48	6.7	3.20	65.00	44	6.7	
March 30	1.00	29.00	34	6.7	*				1.20	34.00	36	6.7	
					1 ATI	E SEASON							
April 27	 *				2.40	170.00	34	6.7	2.40	84.00	36	6.7	
April 3	1.00	12.00	34	6.7	3.00	66.00	36	6.7	.10	4.40	52	6.6	
April 5	1.00	49.00	40	6.7	1.00	49.00	40	6.7	.10	2.30	46	6.7	
April 6	1.00	100.00	52	6.7		70.00	36	6.7	3.40	4.20	50	6.7	
April 10	1.00	820.00	60	6.7	6.30		77						
April 11/		490.00	56	6.7	.30	200.00	60	6.7	1.0	22.00	58	6.7	
			<u> </u>		EARI	LY SEASON							
1966:	T												
March 10	<.01	.67	40	6.5	*				.06	.27	42	6.2	
March 11	.01	.30	40	6.2	*				.01	.44	40	6.2	
March 13	.08	.17	46	6.9	.55	17.00	46	7.1	.01	.05	40	6.9	
March 14	.20	2.10	40	6.9	.10	3.80	40	6.9	.19	.30	38	6.7	
March 15	.69	.90	40	6.7	.22	1.10	50	6.6	.09	1.80	38	6.7	
March 17	.06	1.00	43	6.2	.05	1.00	46	6.2	.02	1.00	50	6.5	
March 18	.12	.50	58	6.9	.04	1.00	48	6.2	.<.01	1.00	60	6.5	

					MIDSEASON					*.	
March 19	.10	2.20	60	6.9	*			*			**************************************
March 20 March 23	.09				.15 1600.00	60	6.5	*			
included 257	1 .09	2300.00	56	7.0	.19 2800.00	60	6.8	.05	202.00	52	7.0
					LATE SEASON						
April 1	8.70		50	6.9	*			*			
April 2 April 3	1.90		52	6.9	.70 320.00	40	6.5	*			
April 4	.13		_		.40 1100.00	38	6.9	.08	75.00	40	6.9
April 5	.06	16.00	40	6.9	.60 54.00	46	6.9	.20	13.00	38	7.0
April 6	1.20	18.00 97.00	46	7.0	.18 70.00	46	7.0	.06	16.00	42	6.7
April 7	1.70	110.00	44	6.9	*			*		74	0.7
April 8	.30	1800.00	46 48	6.9	.04 62.00	46	6.9	.60	40.00	46	6.9
April 9	2.30	180.00	46	6.9	.40 220.00	40	6.9	2.20	250.00	46	6.5
April 10	3.70	140.00	48	6.9 5.9	.20 940.00 3.20 520.00	42	6.9	*			
				<u> </u>	3.20 520.00	40	5.9	*			
1067					EARLY SEASON						
1967:											
March 23	.70	4.10	36	7.0	.27 1.40	40	7.0	.13	24		
March 24	.38	2.80	40	7.0	.51 1.10	40	7.0	.43	.24	36	7.0
March 25 March 26	.06	1.40	40	7.0	.08 .71	50	7.0	.01	.65 .10	40	7.0
March 277	.27 .10	2.10	40	7.0	.01 2.50	40	7.0	.03	.58	50	7.0
March 2//	.10	2.70	46	7.0	.20 95.00	44	7.0	.15	4.00	59 44	7.0 7.0
					MIDSEASON						
March 30	,20	2.10	58	7.0	.20 2.20						
March 31	<.01	4.40	50	7.0	.20 2.20 .26 36.00	57	7.0	.22	1.10	58	7.0
• • • • • • • • • • • • • • • • • • •						60	7.0	.28	2.80	60	7.0
Annet 1 /					LATE SEASON						
April 4	<.01	1.70	42	7.0	3.80 290.00	42	7.0	.22	230.00	60	7.0

^{*}Tank not in service.

 $[\]mathcal{T}_{\mathrm{Tank}}$ sanitized before this run.

TABLE 4.--Microbiological contamination levels of sap in the stationary tanks used with the plastic tubing sap collection system, 1965, 1966, and 1967

Date		Tank No.	1			Tank No.	2			Tank No.	3	in the second of
sample obtained	Yeast	Bacteria	Temp.	pН	Yeast	Bacteria	Temp.	рН	Yeast	Bacteria	Temp.	рН
	cells/n	al. x 10 ⁻³	• F		cells/i	ml. x 10 ⁻³	•F		cells/m	1. x 10 ⁻³	• _F	
a version en la general					EA	RLY SEASON						
1965:												
March 4	0.02	0.05	72	7.0	0.01	0.30	71	7.1	0.01	0.14	70	6.9
March 10	<.01	.01	54	6.7	<.01	.10	58	6.7	.01	.10	60	6.7
March 14	*				.10	.14	64	6.6	.24	.10	56	6.7
March 15	.31	6.20	66	6.4	<.01	.10	64	6.4	.01	.30	64	6.5
March 17	.12	8.90	45	6.4	.01	.02	45	6.7	.01	.47	45	6.7
March 18	.10	13.00	50	6.5	<.01	.01	56	6.6	.02	.70	52	6.4
March 19	.10	14.00	60	6.4	<.01	.10	62	6.7	.01	1.30	60	6.7
The control was a business of					M	IDSEASON			and the second			
March 23	.10	620.00	62	6.7	<.01	.01	58	6.7	.03	.08	60	6.4
March 26		1,100.00	50	6.6	<.01	.01	52	6.6	.01	.11	52	6.6
March 28		1,900.00	62	6.5	<.01	.10	68	6.6	.01	.11	62	6.5
					LA	TE SEASON						
April 1	1 10.00	520.00	62	6.4	.02	.17	60	6.6	.01	.01	62	6.5
April 2		1,300.00	61	6.5	.10	.39	60	6.6	.43	.04	63	6.5
April 3		1,300.00	62	6.6	.01	.77	54	6.6	.10	.05	54	6.6
April 4		2,200.00	62	6.5	.05	.79	62	6.5	.12	.14	62	6.5
April 5		2,600.00	72	6.4	.32	1.20	70	6.4	.10	.50	72	6.4
April 6		2,700.00	70	6.4	.10	2.70	70	6.4	.10	.60	70	6.4
April 11		5,500.00	60	6.4	2.70	33.50	62	6.4	1.80	19.00	62	6.7
					EA	RLY SEASON						
1966:												
March 5					.01	.08	60	6.5	<.01	.50	58	5.9
March 11	<.01	<.01	53	6.6	.01	.02	58	6.6	.02	.27	55	6.6
March 12	<.01	.20	57	6.9	<.01	.01	55	6.9	<.01	.31	55	6.9
March 14	<.01	.50	64	6.5	<.01	.05	64	6.2	<.01	.69	66	6.2
March 15	*				.02	.09	46	6.7	.10	1.20 4.50	48 36	6.7 6.7
March 16	<.01	.30	35	6.7	.01	.06	35	6.9	<.01	4.30	20	0.7

March 17 March 18	<.01 <.01		35 47	6.9 5.9	<.01 .01	.10 1.70	39 69	6.5 5.9	<.01 .10	10.00 12.00	37 64	6.2 5.9
						IDSEASON				12.00		3.9
March 26	1 20	12.00	~									
March 29	.20		34	6.5	.20	.50	32	6.9	.05	1,400.00	38	6.9
Platell 23	. 30	210.00	34	6.5	.14	11.00	34	6.5	.12	3,300.00	35	6.5
					LA'	TE SEASON						
April 2	.03	4.00	34	6.5	.01	4.20	35	6.5	.02	150.00	38	6.9
April 3	.02	13.00	37	6.2	.04	10.00	38	6.2	.04	360.00	43	6.6
April 4	.25	14.00	50	6.5	.30	10.00	53	6.5	.10	760.00	53	6.5
April 5	.60	34.00	42	6.9	.30	12.00	42	7.0	.70	400.00	40	6.9
April 6	.80	64.00	43	6.9	.31	15.00	44	6.9	3.10		43	6.9
April 7	1.40	100.00	58	6.9	.60	71.00	56	6.9	2.80	1,100.00	55	6.9
April 8	1.60	160.00	42	6.9	1.80	140.00	41	6.9	3.70	890.00	41	6.9
April 9	.90	640.00	41	6.9	3.80	110.00	41	6.9	4.10	2,000.00	41	6.9
April 10	1.20	2,100.00	45	6.1	9.00	540.00	42	6.9	4.00	2,100.00	44	5.9
					EAI	RLY SEASON						
.967:				1					<u> </u>			
March 12	<01	3.00	41	6.8	<.01	.02	43	6.9	<.01	.02	40	6.8
March 13	.10	1.70	34	6.8	.02	.01	35	6.8	<.01	.01	32	7.0
March 14	<.01	1.00	38	6.7	.10	.02	36	7.0	<.01	.01	38	7.0
					М	DSEASON	-					
March 23	<.01	.05	34	6.9	<.01		~~~		. 01			
March 24	<.01	.10	33	6.8	.13	.01 .04	34 35	6.9	<.01	.02	35	6.9
March 25	.01	.01	37	6.9	.09	.07		6.7	<.01	.01	36	7.0
March 26	<.01	.02	37	6.7	.09	.07	44 46	6.8	<.01	.01	47	7.0
March 27	.04	.02	53	6.8	.01	.42	53	6.7 6.9	<.01 <.01	.01 .02	46 50	6.8
March 30	.02	.10	56	6.8	.46	45.00	58	7.0	.01	.02	50 63	6.9
March 31	.25	.20	74	6.7	1.00	47.00	67	7.0	<.01	.05	63 72	6.9 7.0
						E SEASON		7.0	01	•03	14	7.0
April 4	.02	4.90		(0								
April 5	<.01	4.90 5.80	61 77	6.9 7.0	.07	120.00	57	6.9	.01	.25	60	7.0
43WLLL J 1	UI	2.00	11	/.U	1.20	390.00	54	7.0	.04	.80	72	6.9

*No sap.